

Appl. No. 09/972,916

Response to the Restriction/Election Requirement dated April 21, 2004

Reply to Office Action of February 25, 2004

**Amendments to the Specification:**

Please replace paragraph [0002] with the following amended paragraph:

[0002] The present application incorporates by reference a file named: US 1292-01 Replacement Sequence Listing 4-21-04, including SEQ ID NO.: 1, SEQ ID NO.: 2, SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, [[and]] SEQ ID NO.: 6, SEQ ID NO.: 7, SEQ ID NO.: 8, SEQ ID NO.: 9, SEQ ID NO.: 10, SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13, and SEQ ID NO.: 14 provided herewith on a diskette, created on ~~November 21, 2004~~ April 21, 2004 and containing [[3,584]] 4,364 bytes. The information recorded on the diskette is identical to the written sequence listing provided herein.

Please replace paragraph [0089] with the following amended paragraph:

[0089] Base pairs -324 - +96 of the rat IGFBP-1 promoter were PCR amplified from p-930bpcat (Reference 44), and the product ligated into a TA-cloning vector, pCRII™ (Invitrogen, Carlsbad, Ca.). The primers were as follows, novel restriction sites (*Mlu*-I in the forward primer, and *Nhe*-I in the backward primer) are underlined: forward; 5'-GCGACGCGTCCCTTAGGTATTCCTTGAGT-TCGG-3' (SEQ ID No.: 7), backward; 5'-GC GGCTAGCTAGTAGCGGAAGTGGTGGTTCACAG-3' (SEQ ID No.: 8).

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A 526 bp *Kpn*-I / *Xho*-I restriction fragment was then directionally inserted into the luciferase expression vector, pGL2Basic (Promega, Madison, WI), to create p-324-+96BP-1Luc. The p(GIRE)<sub>n</sub>BP-1Luc plasmids were constructed by inserting copies of the glucose responsive element from the rat L-PK gene into the IGFBP-1 promoter. Oligonucleotides corresponding to 50 bp of the positive (5'-GGCGCACGGGGCACTCCGTGGTCTGGACTCTGGCCCCAGTGT-A-3' -SEQ ID No.: 9) and negative (5'-ATGTACACTGGGGGCCAGAGTCCAGGAACCACGG-GAG-TGCCCGTGCGCCC-3' -SEQ ID No.: 10) strands of the rat L-PK GIRE sequence were annealed, multimerized, and size fractionated by polyacrylamide electrophoresis. DNA representing a sequence of GIRE multimers, from one to four, was isolated from excised bands, and blunt-ended by treatment with Klenow. Insertion of GIRE multimers into *Eco*/CR-I restricted p-324-+96BP-1Luc removed all IGFBP-1 sequences 5' to bp -114, and resulted in placement of GIRE sequences immediately 5' to the IGFBP-1 insulin responsive region. Sequencing of resultant plasmid constructs unexpectedly revealed uniform multimerization of all GIRE elements in a head-to-tail orientation. Blunt-end ligation with p-324-+96BP-1Luc consequently produced constructs in which all GIRE's were either in the native, or reverse orientation.

Please replace paragraph [0098] with the following amended paragraph:

[0098]        Immediately upon sacrifice livers of treated and control animals

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were flash-frozen in liquid nitrogen, and stored at -70<sup>0</sup>C. Total RNA (5µg), obtained by using Trizol (Gibco BRL, Gaithersburg, MD) per manufacturer's instructions, was subjected to reverse transcription using an oligo-dT<sub>17</sub> primer, recombinant RNAsin, and Moloney- Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) (all Promega, Madison,WI). M-MLV RT was inactivated at 95<sup>0</sup>C, and PCR reactions were performed using a Gene Amp PCR System 9600 thermal cycler (Perkin Elmer, Norwalk, CT), and AmpliTaq DNA Polymerase (Perkin Elmer, Norwalk, CT). The cDNA-mixture was allowed to react for 19 (GAPDH), or 22 (insulin) cycles. Primers used for human insulin were 5'-ACCATGGCCCTGTGGATGCGC-3' (SEQ ID No.: 11) (forward), and 5'-CTAGTTGC-AGTAGTTCTCCAG-3" (SEQ ID No.: 12) (reverse). Primers for GAPDH were 5'-CTGGTCATCAATGGGA-AAC-3' (SEQ ID No.: 13) (forward), and 5'-CAAAGTTGTCATGG-ATGACC-3' (SEQ ID No.: 14) (reverse).